ASSESSMENT OF CAPTIVE BROODSTOCK TECHNOLOGY 9305600

SHORT DESCRIPTION:
Generate information needed to overcome some of the barriers that limit the yield of viable offspring from Pacific salmon broodstock reared in captivity; evaluate some of the genetic consequences of captive broodstock programs.

SPONSOR/CONTRACTOR: NMFS
National Marine Fisheries Service
Dr. Penny Swanson, Physiologist
2725 Montlake Boulevard East, Seattle, WA 98112-2097
206/860-3282 pswanson@sci.nwfsc.noaa.gov

SUB-CONTRACTORS:
University of Washington, University of Idaho, U.S. Geological Survey (USGS)

GOALS

GENERAL:
Maintains biological diversity, Maintains genetic integrity, Increases run sizes or populations

ANADROMOUS FISH:
Research, M&E

NPPC PROGRAM MEASURE:
7.4D.1

RELATION TO MEASURE:
The project undertakes research that 1) develops genetically sound methods of obtaining and breeding broodstock to ensure genetic stability and gamete quality, 2) models genetic consequences of captive broodstock programs, 3) develops captive broodstock culture systems that minimize loss of fish, 4) evaluates and compare fish husbandry techniques, 5) evaluate fish health problems, and 6) investigates fish reproductive and non-reproductive physiology.

OTHER PLANNING DOCUMENTS:
National Marine Fisheries Service Snake River Salmon Recovery Plan; 4.1.c.

TARGET STOCK	LIFE STAGE	MGMT CODE (see below)
Hood Canal coho salmon	All	W
White River Sp. chinook salmon	All	W
Dungeness Sp. chinook salmon	All	W
West Fork of Yankee Fork Sp/Su chinook salmon	All	W
East Fork Salmon River Sp/Su chinook salmon	All	W
Lemhi River Sp/Su chinook salmon	All	W
Catherine Creek Sp/Su chinook salmon	All	W
Lostine Creek Sp/Su chinook salmon	All	W
Sacramento Wn. chinook salmon	All	W
Redfish Lake sockeye salmon	All	W

BACKGROUND

HISTORY:
In response to Task 4.1.c in the NMFS Proposed Recovery Plan and to Measure 7.4D.1 in the NPPC Fish and Wildlife Program, this research project develops information needed to overcome some of the problems that limit the yield of viable offspring from Pacific salmon stocks reared in captivity, and assesses some of the genetic consequences of captive broodstock programs. The high fecundity of Pacific salmon, coupled to their potentially high survival in protective culture, affords the opportunity for captive broodstocks to produce large numbers of juveniles in a single generation for supplementation of natural salmon populations. However, numerous problems have been encountered by captive broodstock programs including poor survival of

adults to maturity and poor quality gametes. Furthermore, the reproductive success of captively-reared fish compared to wild fish, and the genetic consequences of captive rearing are largely unknown. Fish culture methods need improvement to minimize changes in the genetic, physiological, and behavioral characteristics of the fish. This project is conducted by a multidisciplinary research team that includes scientists from NMFS, University of Washington, University of Idaho, WDFW, USGS, the Suquamish Tribe, and the NW Indian Fisheries Commission. Cooperation of scientists with a wide range of expertise in one location increases the cost-effectiveness of this research and integrates various scientific disciplines into a sensible, comprehensive program.

BIOLOGICAL RESULTS ACHIEVED:

This study does not directly alter habitat or produce fish for release to enhance depressed stocks. Surrogate stocks of fish are reared as part of experiments to test specific hypotheses and to ultimately develop improved methods for captive rearing of wild stocks of Pacific salmon. Below are results obtained during 1994-1995 that are directly applicable to captive broodstock programs.

* Comparison of Lake Wenatchee sockeye salmon broodstock reared in fresh water throughout the lifecycle to those reared for a period in filtered seawater indicated that fish reared in fresh water for the complete life-cycle had higher survival to spawning, were larger in body size, and had larger eggs. Gamete quality, in terms of fertilization rates and survival of offspring to the eyed-stage did not differ between these two groups. Results from this study suggest that broodstock programs which rear fish in fresh water throughout the life cycle would have higher yield of viable offspring that those reared for a period in seawater.
*Sockeye and coho salmon fry reared on live food (Artemia) had higher growth than those reared on commercially available diets.
* Implants containing gonadotropin-releasing hormone analogue successfully induced ovulation and milt production in coho and sockeye salmon broodstock without affecting gamete qualtity. The dosage of hormone, mode of administration, and timing of response were determined for both species. This technology was successfully used to induce spawning in a few Redfish Lake sockeye salmon broodstock and one wild male, and in wild Sacramento River winter-run chinook salmon captured for the captive broodstock program in California.
* Comparison of juvenile chinook salmon reared on five levels of dietary fat and similar growth rates indicated that the percentage of early male maturity (as 2 year-old fish) was correlated with body fat levels. These data suggest that reducing fat levels in captively reared fish by reducing dietary fat may reduce the rate of early male maturation.

PROJECT REPORTS AND PAPERS:

Flagg, T. A. and Mahnken, C.V. W. 1995. An Assessment of the Status of Captive Broodstock Technology for Pacific Salmon. Report to the Bonneville Power Administration, Contract DE-AI79-93BP55064, 295p.

ADAPTIVE MANAGEMENT IMPLICATIONS:

The information generated from this project is being directly applied to captive broodstock programs to aid recovery of endangered and threatened stocks of Pacific salmon. The research in this project has been designed to address specific problems incurred by ongoing captive broodstock programs, or to evaluate unknown risks of captive rearing versus captive breeding strategies. The results from the genetic studies will be used to determine the genetic consequences of captive broodstock programs for the viability of natural salmon populations, thus evaluating the risks of captive broodstock programs as rehabilitative tools.

PURPOSE AND METHODS

SPECIFIC MEASUREABLE OBJECTIVES:

The overall objective of this project is to develop standard, efficient hatchery practices for rearing captive Pacific salmon broodstock that yield the greatest number of high-quality offspring (those that are as similar to the founder stock as possible). Specific objectives include:•

* develop broodstock diets that improve egg quality
* develop diets and rearing regimes that minimize early male maturity in chinook salmon•
* develop technology to control spawning time in Pacific salmon species to minimize loss of gametes due to pre-spawning mortality and to synchronize spawning in male and female broodstock
* test rearing temperatures for adult sockeye salmon broodstock that optimize survival of adults to spawning and do not impair gamete quality
* develop anti-bacterial therapies for reducing mortality due to bacterial kidney disease
* determine reproductive success of captively reared adult coho salmon

* quantify genetic effects of inbreeding and outbreeding depression

BIOLOGICAL NEED:

While basic fish husbandry techniques are well established and widely used for rearing juvenile salmonids from gametes collected from returning adults and domesticated stocks of salmonids in the commercial aquaculture industry, numerous problems have persisted when rearing wild stocks of Pacific salmon in captivity throughout the life-cycle. These problems include poor survival of adults to spawning, poor quality gametes, and abnormal seasonal timing of spawning. The success of captive broodstock programs for stock restoration purposes is largely dependent on producing a high yield of offspring that do not differ substantially from the founder stock in genetics, behavior, appearance, or physiology. Solutions to the problems encountered by broodstock programs are needed to maximize the effectiveness of these programs as rehabilitative tools. In addition, the reproductive success of captively reared fish must be evaluated to determine if release of captively reared adults is a viable strategy.

HYPOTHESIS TO BE TESTED:

This project tests a number of specific null hypotheses:

1. Rearing sockeye salmon broodstock in fresh water or seawater does not affect survival of adults to spawning, timing of maturation, age of maturity, qualtity of gametes, or viability of offspring
2. Timing of transfer of seawater-reared broodstock to fresh water prior to spawning does not affect survival or reproductive performance of broodstock. •
3. Rearing temperature (8-12 0C) does not affect development, reproductive performance, timing or age of maturity, immune function, or disease resistance in captively-reared sockeye salmon.•
4. Reproductive performance of adult sockeye salmon fed diets enhanced with elevated levels of vitamins, dietary protein, or carotenoid do not differ from fish fed commercially available broodstock diets.
5. Growth, condition, and behavior of juvenile salmon fed live food do not differ from that of salmon fed commercially available diets.
6. Disease-challenged fry fed either untreated live foods (e.g., Artemia) or those which have been treated with antibiotics do not differ in rates of infection or mortality due to disease (e.g., bacterial kidney disease) .
7. Sockeye salmon adults (2-3 years of age) fed diets with elevated fat levels do not differ from those fed commercially available broodstock diets or diets with reduced fat levels in terms of age of maturity or gamete quality.
8. Increased growth rates or rates of fat deposition during critical periods (autumn or spring) of the life-cycle do not alter the number of male chinook salmon maturing as 1 or 2-year old fish.
9. The effectiveness of azithromycin as antibacterial therapy to reduce mortality due to bacterial kidney disease does not differ from erythromycin.
10. The effectiveness of gonadotropin-releasing hormone analogue in inducing ovulation or milt production is not altered by the timing of administration, dose, or mode of administration.
11. The quality of gametes is not affected by artificial induction of ovulation or milt production with gonadotropin-releasing hormone.
12. Reproductive success of wild and captively-reared adult coho salmon do not differ.
13. Inbreeding does not affect body shape, growth, age of maturity, fecundity, egg size, disease resistance, survival of fish to spawning, or rate of return in chinook salmon.
14. Outbreeding depression does not occur in closely related populations (e.g., wild fish and hatchery fish that are derived from them) of Pacific salmon.

METHODS:

Experiments are listed briefly in the order used for testable hypotheses.

1. 1990- and 1991-brood Lake Wenatchee sockeye salmon were reared from fertilized eggs to spawning adults in either fresh water (University of Washington, Big Beef Creek Field Station), filtered and UV-treated seawater, or net-pens in seawater (NMFS Manchester Marine Experimental Station). Adult fish were reared in tanks or net-pens (3 tanks/treatment) to maturity. Rates of survival, growth, age of maturity, gamete quality, and survival of offspring were evaluated. Data are being analyzed by ANOVA and ANCOVA.
2. 1993-Brood Lake Wenatchee sockeye salmon reared in tanks supplied with fresh water through smoltification and seawater net-pens through maturity as 4-year old fish will be transferred to freshwater tanks 1-4 months prior to historical spawning time for this stock (mid-September). Survival of fish to spawning, timing of spawning, gamete quality, and survival of offspring will be compared. Data will be analyzed by ANOVA.
3. Lake Wenatchee sockeye salmon (1994-brood) are being reared in freshwater tanks (3 tanks/ treatment) under one of three temperature regimes: constant (8 0C), constant (12 0C), or seasonally fluctuating temperature (8-12 0C). Rates of growth,

development, and maturation are being monitored by destructive monthly subsampling. Immune function and disease resistance are monitored twice annually (spring and fall) from 1996 through 1998. Age of maturity, timing of smoltification and maturation, gamete quality, immune function (both humoral and cellular- mediated immunity) will be compared among treatments. Statistical analysis will include ANOVA, ANCOVA, regression, and non-parametric tests.
4. Lake Wenatchee sockeye salmon (1993-brood year) fed one commercial and two test-diets (3 tanks/ treatment) are being reared in freshwater tanks throughout the life-cycle. Two test diets include enhanced vitamins and supplemented carotenoids, or elevated fat (22%). Growth, body composition, and gonadal development are monitored with destructive subsampling. Age of maturity (3-5 years of age), growth, egg size, gamete quality, and offspring survival will be evaluated by ANOVA and ANCOVA.
5. Growth in Lake Wenatchee sockeye salmon fry (1994- and 1995-brood fish) fed either live Artemia (adult or nauplii), Biodiet, or dried adult Artemia (3 tanks per treatment) are being compared during a 6-week feeding trial, and after all fish are changed to a commercial diet (Biodiet).
6. Lake Wenatchee sockeye salmon fry (1995-brood) are fed either Artemia or Artemia treated with erythromycin (3 tanks/treatment). Fry from each treatment are challenged with the causative agent of bacterial kidney disease (BKD), and infection with BKD is monitored by ELISA and mortality due to BKD is determined.
7. Test of elevated fat level is included in experiment outlined in #4.
8. A 2 x 2 factorial design experiment to test the effects of growth rate versus body fat levels on early male maturity is being conducted with White River spring chinook salmon (1995-brood). Fish are reared on high ration and either high- or low-fat diets, or low ration and high- or low-fat diets. Additional treatments are included during fall 1996 and spring 1997: subgroups of low-fat high ration fish are fed high-fat diets during either the fall or spring to increase body fat levels during supposed critical periods of development. Growth, body composition, smoltification, gonadal development, and number of males maturing as 1- or 2-year old fish are compared among the treatments.
9. Juvenile sockeye salmon are fed diets containing either no antibiotic, or one of three doses of either erythromycin or azithromycin (2 tanks per treatment). Fish are challenged with the causative agent of BKD, and mortality due to BKD is monitored.
10 &11. Sexually mature coho salmon (Domsea broodstock) or Lake Wenatchee sockeye salmon (wild fish captured during spawning migration during autumn 1995), were treated (10 fish/treatment) with one of two doses of gonadotropin-releasing hormone analogue (25 or 75 æg) in either biodegradable microspheres or pelleted implants 1 month prior to the normal spawning period for these stocks. Timing of ovulation or sperm production, egg size, milt volume, sperm motility, fertilization rates, and survival of offspring to eyed-stage were monitored and compared among the treatment groups. Data were analyzed by ANOVA and ANCOVA.
12. Reproductive success of two geographically proximate populations of wild and captively-reared coho salmon are being evaluated by observing mating in a closed-stream environment and DNA fingerprinting of all adults used in the experiment and fry produced. In addition, gamete quality and survival of offspring produced by wild and captively reared adults are compared in a 3 x 3 factorial designed mating matrix (wild-wild versus wild-captive, versus captive-captive crosses).
13. Inbreeding is being evaluated in a conventional nested breeding design using Grover's Creek fall chinook salmon and genetic crosses with 100 full-sib families and 30 half-sib families. Life history characters will be evaluated in F1 and F2 offspring.•
14. Outbreeding depression experiments will start in October, 1997. The experiments will employ three populations of coho salmon (two hatchery and one wild) and their first and second generation hybrids to test different genetic models of population divergence. These tests will be used to detect whether outbreeding depression occurs and if it exists, to determine its genetic mechanism.

PLANNED ACTIVITIES

SCHEDULE:

PROJECT COMPLETION DATE:
2001

CONSTRAINTS OR FACTORS THAT MAY CAUSE SCHEDULE OR BUDGET CHANGES:
There are no known risks of this research project to natural salmon populations.

OUTCOMES, MONITORING AND EVALUATION

SUMMARY OF EXPECTED OUTCOMES

Expected performance of target population or quality change in land area affected:
The overall goal of this project is to develop diets, rearing regimes, hatchery practices, and drug therapies that improve survival of adults to spawning, gamete quality, and viability of offspring that can be applied to captive broodstock programs for depressed stocks of Pacific salmon.

Contribution toward long-term goal:
Information on influence of numerous variables on growth, survival, and reproduction of captive sockeye, chinook and coho salmon. Measurement of genetic consequences of captive broodstock programs. Developing effective broodstock technology will give a "jump-start" to recovery of severely threatened and endangered salmonid stocks.

Coordination outcomes:
1994-1995
1) Completed literature review and identified the most critical problems in rearing Pacific salmon and their probable causes: a) low survival of fish to spawning; b) inappropriate timing of sexual maturation; and c) poor quality gametes. Causes include inappropriate rearing conditions, diets, and growth regimes, and lack of disease therapy. The literature review confirmed that little research has been done on genetic consequences of captive broodstock programs on Pacific salmon.
2) Initiated experiments on: a) effects of rearing environment and nutrition on reproductive performance in sockeye salmon; b) effects of rearing temperature on growth, reproductive performance, and immune function in sockeye salmon; c) effects of diet and growth regime on precocious maturation in chinook salmon; d) development of new drug therapies for bacterial kidney disease; and e) quantitative genetic problems associated with inbreeding and inbreeding depression. Initial results were obtained for (a) above in winter 1996.

MONITORING APPROACH
(See Methods section)

Data analysis and evaluation:
Standard statistical methods will be used as appropriate (Zar 1984). These include both parametric and non-parametric analyses, ANOVA, multiple range testing (Fisher PLSD), linear and nonlinear regression, and chi-square.

Information feed back to management decisions:
Progress reports of the research findings will be presented at seminars, workshops, scientific meetings, processed reports, and publications in the peer-reviewed literature.

EVALUATION
Publication of findings, acceptance of findings by the scientific community. Improved success of on-going captive broodstock programs.

Incorporating new information regarding uncertainties:
The project is critically reviewed internally and externally by scientists and administrators.

Increasing public awareness of F&W activities:
All publications and presentations acknowledge the region's (BPA) efforts and support.

RELATIONSHIPS

RELATED BPA PROJECT	RELATIONSHIP
9604400 Grande Ronde Basin Spring Chinook Captive Broodstock Program Capitol Construction Component	Application of research findings to improve captive brood program

9107200 Idaho Department of Fish and Game Sockeye Salmon Captive Broodstock Program	Application of research findings to improve captive brood program
9700100 Captive Rearing Initiative for Salmon River Chinook Salmon	Application of research findings to improve captive brood program
9202200 Wild Smolt Behavior/Physiology (esa)	Collaboration on effects of growth rate on smolt quality and precocious sexual maturation
9204000 Redfish Lake Sockeye Captive Broodstock Program	Application of research findings to improve sockeye captive brood program

RELATED NON-BPA PROJECT	**RELATIONSHIP**
Endocrinology of salmon reproduction/ U.S. Dept. Agriculture	Fundamental research to improve understanding of salmon reproduction

OPPORTUNITIES FOR COOPERATION:

This project involves and depends on the cooperation of state (WDFW) and federal agencies (NMFS, U.S. Geological Survey), universities (University of Washington and University of Idaho), private non-profit organizations (Long Live the Kings) and tribes (Suquamish tribe). Staff and scientists from all of these entities actively participate in various aspects of the research described above.

COSTS AND FTE

1997 Planned: $1,000,000

FUTURE FUNDING NEEDS:

FY	$ NEED	% PLAN	% IMPLEMENT	% O AND M
1998	$1,250,000		100%	
1999	$1,250,000		100%	
2000	$1,250,000		100%	
2001	$1,250,000		100%	
2002	$1,250,000		100%	

PAST OBLIGATIONS (incl. 1997 if done):

FY	OBLIGATED
1993	$221,000
1994	$1,100,000
1995	$1,101,100
1996	$979,000
TOTAL:	$3,401,100

Note: Data are past obligations, or amounts committed by year, not amounts billed. Does not include data for related projects.

OTHER NON-FINANCIAL SUPPORTERS:

Long Live the Kings

1997 OVERHEAD PERCENT: 45.6% of total direct labor costs

HOW DOES PERCENTAGE APPLY TO DIRECT COSTS:

45.6% of total direct labor costs

CONTRACTOR FTE: 9

SUBCONTRACTOR FTE: 8